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[54] **NUCLEIC ACID ENCODING HUMAN GPR14 RECEPTOR**

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[51] **Int. Cl.**[6] **C12N 15/12**; C07K 14/705

[52] **U.S. Cl.** **435/69.1**; 435/252.3; 435/254.11; 435/320.1; 435/325; 536/23.5

[58] **Field of Search** 435/69.1, 325, 435/252.3, 254.11, 320.1; 536/23.5

[56] **References Cited**

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Yamada, Y. et al., "Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylyl cyclase", *Molecular Endocrinology*, 6(12): 2136–2142, 1992.

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[57] **ABSTRACT**

Human GPR14 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing Human GPR14 polypeptides and polynucleotides in the design of protocols for the treatment of infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parlinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others and diagnostic assays for such conditions.

19 Claims, 3 Drawing Sheets

NUCLEIC ACID ENCODING HUMAN GPR14 RECEPTOR

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to G-Protein coupled receptor, hereinafter referred to as Human GPR14. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers, e.g., cAMP (Lefkowitz, Nature, 1991, 351:353–354). Herein these proteins are referred to as proteins participating in pathways with G-proteins or PPG proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and dopamine (Kobilka, B. K., et al., Proc. Natl Acad. Sci., USA, 1987, 84:46–50; Kobilka, B. K., et al., Science, 1987, 238:650–656; Bunzow, J. R., et al., Nature, 1988, 336:783–787), G-proteins themselves, effector proteins, e.g., phospholipase C, adenyl cyclase, and phosphodiesterase, and actuator proteins, e.g., protein kinase A and protein kinase C (Simon, M. I., et al., Science, 1991, 252:802–8).

For example, in one form of signal transduction, the effect of hormone binding is activation of the enzyme, adenylate cyclase, inside the cell. Enzyme activation by hormones is dependent on the presence of the nucleotide GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylate cyclase. G-protein was shown to exchange GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

The membrane protein gene superfamily of G-protein coupled receptors has been characterized as having seven putative transmembrane domains. The domains are believed to represent transmembrane a-helices connected by extracellular or cytoplasmic. loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

G-protein coupled receptors have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. The G-protein family of coupled receptors includes dopamine receptors which bind to neuroleptic drugs used for treating psychotic and neurological disorders. Other examples of members of this family include, but are not limited to, calcitonin, adrenergic, endothelin, cAMP, adenosine, muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorant, and cytomegalovirus receptors.

Most G-protein coupled receptors (or otherwise known as 7TM receptors) have single conserved cysteine residues in each of the first two extracellular loops which form disulfide bonds that are believed to stabilize functional protein structure. The 7 transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

Phosphorylation and lipidation (palmitylation or famesylation) of cysteine residues can influence signal transduction of some G-protein coupled receptors. Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the b-adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

For some receptors, the ligand binding sites of G-protein coupled receptors are believed to comprise hydrophilic sockets formed by several G-protein coupled receptor transmembrane domains, said socket being surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form polar ligand binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson et al., Endoc. Rev., 1989, 10:317–331) Different G-protein a-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors have been identified as an important mechanism for the regulation of G-protein coupling of some G-protein coupled receptors. G-protein coupled receptors are found in numerous sites within a mammalian host.

Over the past 15 years, nearly 350 therapeutic agents targeting 7 transmembrane (7 TM) receptors have been successfully introduced onto the market.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to Human GPR14 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such Human GPR14 polypeptides and polynucleotides. Such uses include the treatment of infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hyper-

trophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with Human GPR14 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate Human GPR14 activity or levels.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A, 1B, and 1C show the nucleotide and deduced amino acid sequence of Human GPR 14. SEQ ID NOS: 1 and 2.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"Human GPR14" refers generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said Human GPR14 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said Human GPR14.

"Human GPR14 polypeptides" refers to polypeptides with amino acid sequences sufficiently similar to Human GPR14 sequences, preferably exhibiting at least one biological activity of the receptor.

"Human GPR14 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 or allelic variants thereof and/or their complements.

"Human GPR14 polynucleotides" refers to polynucleotides containing a nucleotide sequence which encodes a Human GPR14 polypeptide or fragment thereof, or a nucleotide sequence which has at least 75.9% identity to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or the corresponding fragment thereof, or a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS—STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1–12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626–646 and Rattan et al., "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48–62.

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CORRECTED PUBLICATION

(54) **NUCLEIC ACIDS, PROTEINS, AND ANTIBODIES**

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See (60) Related U.S. Application Data.

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Related U.S. Application Data

(60) Provisional application No. 60/179,065, filed on Jan. 31, 2000. Provisional application No. 60/180,628, filed on Feb. 4, 2000. Provisional application No. 60/214,886, filed on Jun. 28, 2000. Provisional application No. 60/217,487, filed on Jul. 11, 2000. Provisional application No. 60/225,758, filed on Aug. 14, 2000. Provisional application No. 60/220,963, filed on Jul. 26, 2000. Provisional application No. 60/217,496, filed on Jul. 11, 2000. Provisional application No. 60/225,447, filed on Aug. 14, 2000. Provisional application No. 60/218,290, filed on Jul. 14, 2000. Provisional application No. 60/225,757, filed on Aug. 14, 2000. Provisional application No. 60/226,868, filed on Aug. 22, 2000. Provisional application No. 60/216,647, filed on Jul. 7, 2000. Provisional application No. 60/225,267, filed on Aug. 14, 2000. Provisional application No. 60/216,880, filed on Jul. 7, 2000. Provisional application No. 60/225,270, filed on Aug. 14, 2000. Provisional application No. 60/251,869, filed on Dec. 8, 2000. Provisional application No. 60/235,834, filed on Sep. 27, 2000. Provisional application No. 60/234,274, filed on Sep. 21, 2000. Provisional application No. 60/234,223, filed on Sep. 21, 2000. Provisional application No. 60/228,924, filed on Aug. 30, 2000. Provisional application No. 60/224,518, filed on Aug. 14, 2000. Provisional application No. 60/236,369, filed on Sep. 29, 2000. Provisional application No. 60/224,519, filed on Aug. 14, 2000. Provisional application No. 60/220,964, filed on Jul. 26, 2000. Provisional application No. 60/241,809, filed on Oct. 20, 2000. Provisional application No. 60/249,299, filed on Nov. 17, 2000. Provisional application No. 60/236,327, filed on Sep. 29, 2000. Provisional application No. 60/241,785, filed on Oct. 20, 2000. Provisional application No. 60/244,617, filed on Nov. 1, 2000. Provisional application No. 60/225,268, filed on Aug. 14, 2000. Provisional application No. 60/236,368, filed on Sep. 29, 2000. Provisional application No. 60/251,856, filed on Dec. 8, 2000. Provisional application No. 60/251,868, filed on Dec. 8, 2000. Provisional application No. 60/229,344, filed on Sep. 1, 2000. Provisional application No. 60/234,997, filed on Sep. 25, 2000. Provisional application No. 60/229,343, filed on Sep. 1, 2000. Provisional application No. 60/229,345, filed on Sep. 1, 2000. Provisional application No. 60/229,287, filed on Sep. 1, 2000. Provisional application No. 60/229,513, filed on Sep. 5, 2000. Provisional application No. 60/231,413, filed on Sep. 8, 2000. Provisional application No. 60/229,509, filed on Sep. 5, 2000. Provisional application No. 60/236,367, filed on Sep. 29, 2000. Provisional application No. 60/237,039, filed on Oct. 2, 2000. Provisional application No. 60/237,038, filed on Oct. 2, 2000. Provisional application No. 60/236,370, filed on Sep. 29, 2000. Provisional application No. 60/236,802, filed on Oct. 2, 2000. Provisional application No. 60/237,037, filed on Oct. 2, 2000. Provisional application No. 60/237,040, filed on Oct. 2, 2000. Provisional application No. 60/240,960, filed on Oct. 20, 2000. Provisional application No. 60/239,935, filed on Oct. 13, 2000. Provisional application No. 60/239,937, filed on Oct. 13, 2000. Provisional application No. 60/241,787, filed on Oct. 20, 2000. Provisional application No. 60/246,474, filed on Nov. 8, 2000. Provisional application No. 60/246,532, filed on Nov. 8, 2000. Provisional application No. 60/249,216, filed on Nov. 17, 2000. Provisional application No. 60/249,210, filed on Nov. 17, 2000. Provisional application No. 60/226,681, filed on Aug. 22, 2000. Provisional application No.60/225,759, filed on Aug. 14, 2000. Provisional application No. 60/225,213, filed on Aug. 14, 2000. Provisional application No. 60/227,182, filed on Aug. 22, 2000. Provisional application No. 60/225,214, filed on Aug. 14, 2000. Provisional application No. 60/235,

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(57) **ABSTRACT**

The present invention relates to novel proteins. More specifically, isolated nucleic acid molecules are provided encoding novel polypeptides. Novel polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human polynucleotides and/or polypeptides, and antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to these novel polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting or enhancing the production and function of the polypeptides of the present invention.

Related U.S. Application Data

836, filed on Sep. 27, 2000. Provisional application No. 60/230,438, filed on Sep. 6, 2000. Provisional application No. 60/215,135, filed on Jun. 30, 2000. Provisional application No. 60/225,266, filed on Aug. 14, 2000. Provisional application No. 60/249,218, filed on Nov. 17, 2000. Provisional application No. 60/249,208, filed on Nov. 17, 2000. Provisional application No. 60/249,213, filed on Nov. 17, 2000. Provisional application No. 60/249,212, filed on Nov. 17, 2000. Provisional application No. 60/249,207, filed on Nov. 17, 2000. Provisional application No. 60/249,245, filed on Nov. 17, 2000. Provisional application No. 60/249,244, filed on Nov. 17, 2000. Provisional application No. 60/249,217, filed on Nov. 17, 2000. Provisional application No. 60/249,211, filed on Nov. 17, 2000. Provisional application No. 60/249,215, filed on Nov. 17, 2000. Provisional application No. 60/249,264, filed on Nov. 17, 2000. Provisional application No. 60/249,214, filed on Nov. 17, 2000. Provisional application No. 60/249,297, filed on Nov. 17, 2000. Provisional application No. 60/232,400, filed on Sep. 14, 2000. Provisional application No. 60/231,242, filed on Sep. 8, 2000. Provisional application No. 60/232,081, filed on Sep. 8, 2000. Provisional application No. 60/232,080, filed on Sep. 8, 2000. Provisional application No. 60/231,414, filed on Sep. 8, 2000. Provisional application No. 60/231,244, filed on Sep. 8, 2000. Provisional application No. 60/233,064, filed on Sep. 14, 2000. Provisional application No. 60/233,063, filed on Sep. 14, 2000. Provisional application No. 60/232,397, filed on Sep. 14, 2000. Provisional application No. 60/232,399, filed on Sep. 14, 2000. Provisional application No. 60/232,401, filed on Sep. 14, 2000. Provisional application No. 60/241,808, filed on Oct. 20, 2000. Provisional application No. 60/241,826, filed on Oct. 20, 2000. Provisional application No. 60/241,786, filed on Oct. 20, 2000. Provisional application No. 60/241,221, filed on Oct. 20, 2000. Provisional application No. 60/246,475, filed on Nov. 8, 2000. Provisional application No. 60/231,243, filed on Sep. 8, 2000. Provisional application No. 60/233,065, filed on Sep. 14, 2000. Provisional application No. 60/232,398, filed on Sep. 14, 2000. Provisional application No. 60/234,998, filed on Sep. 25, 2000. Provisional application No. 60/246,477, filed on Nov. 8, 2000. Provisional application No. 60/246,528, filed on Nov. 8, 2000. Provisional application No. 60/246,525, filed on Nov. 8, 2000. Provisional application No. 60/246,476, filed on Nov. 8, 2000. Provisional application No. 60/246,526, filed on Nov. 8, 2000. Provisional application No. 60/249,209, filed on Nov. 17, 2000. Provisional application No. 60/246,527, filed on Nov. 8, 2000. Provisional application No. 60/246,523, filed on Nov. 8, 2000. Provisional application No. 60/246,524, filed on Nov. 8, 2000. Provisional application No. 60/246,478, filed on Nov. 8, 2000. Provisional application No. 60/246,609, filed on Nov. 8, 2000. Provisional application No. 60/246,613, filed on Nov. 8, 2000. Provisional application No. 60/249,300, filed on Nov. 17, 2000. Provisional application No. 60/249,265, filed on Nov. 17, 2000. Provisional application No. 60/246,610, filed on Nov. 8, 2000. Provisional application No. 60/246,611, filed on Nov. 8, 2000. Provisional application No. 60/230,437, filed on Sep. 6, 2000. Provisional application No. 60/251,990, filed on Dec. 8, 2000. Provisional application No. 60/251,988, filed on Dec. 5, 2000. Provisional application No. 60/251,030, filed on Dec. 5, 2000. Provisional application No. 60/251,479, filed on Dec. 6, 2000. Provisional application No. 60/256,719, filed on Dec. 5, 2000. Provisional application No. 60/250,160, filed on Dec. 1, 2000. Provisional application No. 60/251,989, filed on Dec. 8, 2000. Provisional application No. 60/250,391, filed on Dec. 1, 2000. Provisional application No. 60/254,097, filed on Dec. 11, 2000. Provisional application No. 60/231,968, filed on Sep. 12, 2000. Provisional application No. 60/226,279, filed on Aug. 18, 2000. Provisional application No. 60/186,350, filed on Mar. 2, 2000. Provisional application No. 60/184,664, filed on Feb. 24, 2000. Provisional application No. 60/189,874, filed on Mar. 16, 2000. Provisional application No. 60/198,123, filed on Apr. 18, 2000. Provisional application No. 60/227,009, filed on Aug. 23, 2000. Provisional application No. 60/235,484, filed on Sep. 26, 2000. Provisional application No. 60/190,076, filed on Mar. 17, 2000. Provisional application No. 60/209,467, filed on Jun. 7, 2000. Provisional application No. 60/205,515, filed on May 19, 2000. Provisional application No. 60/259,678, filed on Jan. 5, 2001.

NUCLEIC ACIDS, PROTEINS, AND ANTIBODIES

STATEMENT UNDER 37 C.F.R. §1.77(b)(4)

[0001] This application refers to a "Sequence Listing" listed below, which is provided as an electronic document on two identical compact discs (CD-R), labeled "Copy 1" and "Copy 2." These compact discs each contain the following files, which are hereby incorporated in their entirety herein:

Document	File Name	Size in bytes	Date of Creation
Sequence Listing	PTZ02_seqList.txt	152,183	01/15/2001
V Viewer Setup File	SetupDLL.exe	695,808	12/19/2000
V Viewer Help File Controller	v.cnt	7,984	01/05/2001
V Viewer Program File	v.exe	753,664	12/19/2000
V Viewer Help File	v.hlp	447,766	01/05/2001

[0002] The Sequence Listing may be viewed on an IBM-PC machine running the MS-Windows operating system by using the V viewer software, licensed by HGS, Inc., included on the compact discs (see World Wide Web URL: http://www.fileviewer.com).

FIELD OF THE INVENTION

[0003] The present invention relates to novel proteins. More specifically, isolated nucleic acid molecules are provided encoding novel polypeptides. Novel polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human polynucleotides and/or polypeptides, and antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to these novel polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting or enhancing the production and function of the polypeptides of the present invention.

BACKGROUND OF THE INVENTION

[0004] The seven transmembrane receptors (also known as heptahelical, serpentine, or G protein-coupled receptors) comprise a superfamily of structurally related molecules. Possible relationships among seven transmembrane receptors (7TM receptors) for which amino acid sequence had previously been reported are reviewed in Probst et al., *DNA and Cell Biology,* 11(1):1-20 (1992). Briefly, the 7TM receptors exhibit detectable amino acid sequence similarity, and all appear to share a number of structural characteristics including: an extracellular amino terminus; seven predominantly hydrophobic α-helical domains (of about 20-30 amino acids) which are believed to span the cell membrane and are referred to as transmembrane domains TM 1-7; approximately twenty well-conserved amino acids; and a cytoplasmic carboxy terminus. The amino acid similarity among different 7TM receptors ranges from about 10% to more than 80% and receptors which recognize similar or identical ligands generally exhibit high levels of homology. The 7TM receptors can be grouped based on their homology levels and/or the ligands they recognize. For example, the interleukin-8 receptor, the angiotensin II receptor, the thrombin receptor, the endothelin receptors, the N-formyl peptide receptor and the C5a receptor all bind peptide ligands and share 20-40% amino acid similarity.

[0005] 7TM receptors recognize a great diversity of ligands (for example, light, odorants, neurotransmitters, peptide hormones and small molecules) and transduce their signals via heterotrimeric guanine nucleotide-binding proteins (G-proteins) effecting a broad array of biological activities (including visual excitation, olfactory reception, and neurotransmission) through various intracellular enzymes, ion channels and transporters. Signal transduction pathways have been elucidated for rhodopsin (Khorana, *J. Biol. Chem.,* 267:1-4 (1992) and Stryer, *J. Biol. Chem.,* 266:10711-10714 (1991)) and the beta-adrenergic receptors (Dohlman et al., *Ann. Rev. Biochem.,* 60:653-688 (1991)) and are thought to illustrate the pathways utilized by other 7TM receptors. Each 7TM receptor is predicted to associate with a particular G protein at the intracellular surface of the plasma membrane. The binding of the receptor to its ligand is thought to result in activation (i.e., the exchange of GTP for GDP on the α-subunit) of the G protein which in turn stimulates specific intracellular signal-transducing enzymes and channels. Thus, the function of each 7TM receptor is to discriminate its specific ligand from the complex extracellular milieu, and then to activate G proteins to produce a specific intracellular signal. Transmembrane domain-3 (TM3) is believed to be essential in signal transduction (Cotecchia et al., *Proc. Natl. Acad. Sci.,* USA, 87:2896-2900 (1990)). Other regions may be essential for biological activity as well (Lefkowitz, *Nature,* 265:603-604 (1993)).

[0006] Several 7TM receptors have been identified which recognize ligands important for immunological and hemostatic activities (Holmes et al., *Science,* 253:1278-1280 (1991) describes the interleukin 8 receptor (IL8R1) as involved in neutrophil chemotaxis; Sasaki et al., *Nature,* 351:230-233 (1991) reports the angiotensin II receptor (AT2R) is involved in vascular hemostasis). Similarly, the endothelin receptors (Arai et al., *Nature,* 348:730-732 (1990)) regulate vasoconstriction and smooth muscle tone. The C5a receptor mediates chemotaxis, granule enzyme release and superoxide generation in vitro and appears to be involved in anaphylaxis and septic shock in vivo (Gerard and Gerard, *Nature,* 349:614-617 (1991)). Thrombin is also recognized by a 7TM receptor and is a potent activator of platelet aggregation, monocyte chemotaxis, lymphocyte mitogenesis, and mediation of inflammatory responses to vascular injury. The N-formyl peptide (f-met-leu-phe) receptor is responsible for neutrophil chemotaxis and activation (Thomas et al., *J. Biol. Chem.,* 265:20061 (1990)). While these 7TM receptors all have peptide ligands, other 7TM receptors that recognize small organic compounds also

mediate pro-inflammatory activities. For example, the Platelet Activating Factor receptor recognizes a bioactive phospholipid (Honda et al., *Nature,* 349:342-346 (1991)) which causes platelet aggregation and endotoxic shock. The thromboxane A.sub.2 receptor recognizes an arachidonate metabolite which also stimulates vasoconstriction and platelet aggregation and is implicated in stroke and bronchial asthma (Hirata et al., *Nature,* 349:617-620 (1991)).

[0007] Mutations in the third intracellular loop of one 7TM receptor (the thyrotropin receptor) and in the adjacent sixth transmembrane domain of another 7TM receptor (the luteinizing hormone receptor) have been reported to be the genetic defects responsible for an uncommon form of hyperthyroidism (Parma et al., *Nature,* 365:649-651 (1993)) and for familial precocious puberty (Shenker et al., *Nature,* 365:652-654 (1993)), respectively. In both cases the mutations result in constitutive activation of the 7TM receptors. Other studies have shown that mutations that prevent the activation of 7TM receptors are responsible for states of hormone resistance which are responsible for diseases such as congenital nephrogenic diabetes insipidus (See Rosenthal et al., *J. Biol. Chem.,* 268:13030-13033 (1993)). Still other studies have shown that several 7TM receptors can function as protooncogenes and be activated by mutational alteration. See, for example, Allen et al., *Proc. Natl. Acad. Sci. USA,* 88:11354-11358 (1991) which suggests that spontaneously occurring mutations in some 7TM receptors may alter the normal function of the receptors and result in uncontrolled cell growth associated with human disease states such as neoplasia and atherosclerosis. Therefore, mutations in 7TM receptors may underlie a number of human pathologies.

[0008] Phosphorylation and lipidation (palmitylation or farnesylation) of cysteine residues can influence signal transduction of some G-protein coupled receptors. Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the β-adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

[0009] For some receptors, the ligand binding sites of G-protein coupled receptors are believed to comprise hydrophilic sockets formed by several G-protein coupled receptor transmembrane domains, said socket being surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

[0010] G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson et al., *Endoc. Rev,* 1989, 10:317-331) Different G-protein a-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors have been identified as an important mechanism for the regulation of G-protein coupling of some G-protein coupled receptors. G-protein coupled receptors are found in numerous sites within a mammalian host.

[0011] Over the past 15 years, nearly 350 therapeutic agents targeting 7TM receptors have been successfully introduced onto the market. This indicates that these receptors have an established, proven history as therapeutic targets. Thus, there is a clear need for identifying and exploiting novel 7TM receptors, such as those described above. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome. Although structurally related, these receptors will likely possess diverse and multifaceted functions in a variety of cell and tissue types. Receptor type molecules should prove useful in target based screens for small molecules and other such pharmacologically valuable factors. Monoclonal antibodies raised against such receptors may prove useful as therapeutics in antitumor, diagnostic, or other capacities. Furthermore, receptors described here may prove useful in an active or passive immunotherapeutical role in patients with cancer or other immunocompromised disease states. Furthermore, the identification of new 7TM polynucleotides and polypeptides permits the development of a range of derivatives, agonists and antagonists at the nucleic acid and protein levels which in turn have applications in the treatment and diagnosis of a range of conditions such as, as non-limiting examples, infections, neural disorders, cancers, blood and skeletal system disorders, and pain, amongst many other conditions.

SUMMARY OF THE INVENTION

[0012] The present invention relates to novel proteins. More specifically, isolated nucleic acid molecules are provided encoding novel polypeptides. Novel polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human polynucleotides and/or polypeptides, and antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to these novel polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting or enhancing the production and function of the polypeptides of the present invention.

DETAILED DESCRIPTION

[0013] Tables

[0014] Table 1A summarizes some of the polynucleotides encompassed by the invention (including cDNA clones related to the sequences (Clone ID NO:Z), contig sequences (contig identifier (Contig ID:) and contig nucleotide sequence identifier (SEQ ID NO:X)) and further summarizes certain characteristics of these polynucleotides and the